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DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity (Gavage); OECD 408

TEST MATERIAL (PURITY): [redacted]

SYNONYMS: [redacted]

CITATION: [redacted]

SPONSOR: [redacted]

EXECUTIVE SUMMARY:

In a 90-day oral toxicity study [redacted] was administered to 10 Crl:CD(SD) rats/sex/dose by gavage once daily with 0, 0.02, 0.1, and 0.5 mg/kg bw/day (males) and 0, 0.5, 5, and 50 mg/kg bw/day (females). Five additional rats/sex in the control and highest dose groups (0.5 mg/kg bw/day in males; 50 mg/kg bw/day in females) were evaluated after a 13-week recovery period.

No changes in mortality, clinical signs, body weight, reaction to stimuli, motor activity, grip strength, ophthalmology or urinalysis were observed. Slight changes in body weight gain and food consumption were seen only during the recovery period.

In males, statistically significantly lower hemoglobin concentration and hematocrit were seen at 0.1 and 0.5 mg/kg bw/day at the end of the dosing period, with MCHC lower at 0.5 mg/kg bw/day at the end of recovery. Females at 50 mg/kg bw/day exhibited statistically significant decreased prothrombin time at the end of the study. Although not statistically significant, decreases in RBC count, hemoglobin, hematocrit and platelet count and increases in MCV and MCH were also seen in females at 50 mg/kg bw/day.

Several statistically significant changes in clinical chemistry were seen in males, including increased ALT and ALP, total protein and potassium at 0.5 mg/kg bw/day. Albumin and the A/G ratio were higher at both the middle and high doses. AST was increased slightly but more prominently at the end of recovery without statistical significance. In females, statistically significant effects were seen only at 50 mg/kg bw/day but included decreased total bilirubin and increased albumin, total protein and A/G ratios. However, at the end of recovery, total protein was *lower* than controls. An increasing trend in AST and decreasing trend in cholesterol were not statistically significant.

Increased absolute and relative liver weights were seen at 0.1 and 0.5 mg/kg bw/day in males and at 50 mg/kg bw/day in females. Increased relative kidney weights were seen at the same doses.

Generally, gross lesions were seen in the same organs that exhibited histopathological changes and increased weights (primarily the liver and kidney) but a small testis and enlarged epididymis were seen as well.

Histopathological effects in the liver included hepatocyte necrosis and hypertrophy, with eosinophilic granular changes in the cytoplasm of hepatocytes. Fibrosis was seen in one male at the highest dose, and hepatocyte vacuolation was seen in one female at the highest dose at the end of recovery. The hepatocyte hypertrophy may be related to peroxisome proliferation based on the eosinophilic granular changes and increased peroxisomal enzyme activity from a 14-day study but no peroxisomal enzyme assays were conducted in the current study. Kidney effects included proximal tubule hypertrophy, hyaline casts, and basophilic tubules. Diffuse hypertrophy in the adrenal glomerulosa cells was seen in males at 0.5 mg/kg bw/day.

Two rats showed effects on the testis/epididymis at 0.5 mg/kg bw/day (inflammatory cell infiltration was seen in one of the rats and is more non-specific since and similar to infiltration seen in other organs). (Also, the absolute epididymis weight was statistically significantly increased at the end of recovery at this dose.) It is unclear whether these are treatment-related.

The LOAEL is 0.1 mg/kg/day in males, based on liver cell hypertrophy/necrosis, kidney cell hypertrophy, hematology and clinical chemistry changes; the NOAEL for males is 0.02 mg/kg/day. In females, the LOAEL is 5 mg/kg bw/day based on liver and kidney effects and non-statistical increases in enzyme activity, with a NOAEL of 0.5 mg/kg bw/day.

This 90-day oral toxicity study in the Crl:CD(SD) rat is acceptable and satisfies the guideline requirement for a 90-day oral toxicity study (OECD 408) in rats.

COMPLIANCE: Signed and dated GLP and Quality Assurance statements were provided. No Data Confidentiality statement was provided although the data were submitted confidentially.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: [redacted]

Description:	[redacted]
Lot/batch #:	[redacted]
Purity:	[redacted]
Compound stability:	Stable
CAS # of TGAI:	[redacted]

Structure: [redacted]

2. **Vehicle and/or positive control:** Purified water

3. **Test animals:**

Species:	Rat
Strain:	Crl: CD(SD)
Age/weight at study initiation:	5 weeks old; 140-171 g (males) and 124-150 g (females)
Source:	[redacted].
Housing:	One rat/wire cage; two animal rooms used
Diet:	Radiation sterilized pellet diet* fed *ad libitum* [redacted]
Water:	Filtered and UV sterilized tap water *ad libitum*
Environmental conditions:	**Temperature:** 21.2 – 22.6°C **Humidity:** 51.8 – 70.8% **Air changes:** 6-20 times/hr **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	5 days quarantine plus an acclimation period (length not stated)

*Analyzed for contaminants and residual pesticides; all values were within the SOP of the testing laboratory.

B. STUDY DESIGN:

1. **In life dates:** Start = 5 weeks; End of dosing = 18 weeks; End of recovery = 31 weeks

2. **Animal assignment:** Animals were assigned to the test groups noted in Table 1 using a stratified-by-weight randomization procedure to obtain nearly equal mean body weights per dose group.

TABLE 1: Gavage Dosing and Numbers of Rats per Group

Test group	Dose* to animal in mg/kg bw/day (male; female)	# Male	# Female
Control	0 (water)	10; 5 recovery (13 additional weeks)	10; 5 recovery (13 weeks)
Low	0.02; 0.1	10	10
Mid	0.1; 5	10	10
High	0.5; 50	10; 5 recovery	10; 5 recovery

*Administered as 10 mL/kg bw

3. **Dose selection rationale:** The dose levels were selected based on the results of a 28-day oral gavage study in which 0.5 and 5 mg/kg bw/day (males only) and 50 mg/kg (both sexes) resulted in the following effects:
   - low hemoglobin concentration, hematocrit and red blood cell counts;
   - high A/G ratios;
   - livers with increased weights, brown pigment, centrilobular hypertrophy and necrosis of hepatocytes;
   - thyroids with follicular cell hypertrophy; and
   - adrenal glands with glomerulosa cell hypertrophy.

Males also exhibited prolonged prothrombin times as well as high serum urea nitrogen and glucose at all doses. Females exhibited low MCHC, prolonged activated partial thromboplastin time (APTT) and high serum ALT, total protein and albumin at 50 mg/kg bw/day. As noted in the signed consent order dated October 5, 2009, the LOAEL was 0.5 mg/kg bw/day in males and 5 mg/kg-bw/day in females. The NOAEL in females was 0.5 mg/kg bw/day.

4. **Dose preparation and analysis:** Doses were prepared under a non-UV white fluorescent lamp. The test substance (a 50% aqueous solution; 647 mg/mL) was diluted with water to make a 5 mg/mL concentration. The solution was further diluted to make concentrations of 0.5, 0.05, 0.01, and 0.002 mg/mL. Solutions (including vehicle) were divided into brown glass bottles for each dosing day, stored at 3.7 to 6.2 °C, and shielded from light. Solutions were used for dosing within 9 days after preparation.

   Two aliquots of the 5, 0.01, and 0.002 mg/mL solutions were analyzed for stability 4 and 10 days after preparation via HPLC.

   Results:

   Stability analysis: The ratios compared with original concentrations (days 4 and 10) were 104.1 and 103.6% for 0.002 mg/mL; 104 and 103% for 0.01 mg/mL; and 101.4 and 102.8% for 5 mg/mL.

   There was an operational error so that the data of the 'second' of the original (day 0) preparations were used (presumably this refers to the 2nd of the two aliquots tested). Information on stability testing is reported in detail in study [redacted] from the same testing laboratory.

   Concentration analysis: In the 5 final concentrations (0.002, 0.01, 0.05, 0.5, and 5 mg/mL) used for dosing males and/or females, measured concentrations were 103.2 to 105% of nominal concentrations.

5. **Statistics:** *Numerical data* were analyzed by multiple comparison tests. First they were tested by Bartlett's test for homogeneity of variance. When the variance was homogeneous, one-way analysis of variance (ANOVA) was used. If variance was heterogeneous initially, then the Kruskal-Wallis test was used. When a significant difference was seen among groups, the Dunnett (for homogeneous) or Dunnett type (if heterogeneous) multiple comparison test was used to compare means between controls and each dose group. For the recovery groups, an F-test for homogeneity of variance was used. If homogeneous, the Student's t-test was then used. When variance was heterogeneous, the Aspin-Welch t test was used.

   When *categorical* analysis was conducted (urinalysis data), data were tested by Kruskal-Wallis test. If significant differences among groups were seen, the Dunnett type multiple comparison test was then used. The Wilcoxon rank sum test was applied for categorical data during recovery to compare the control with the high dose group.

The Bartlett's, ANOVA, Kruskal-Wallace, and F tests were conducted at the 5% significance level. The other tests were conducted at the two-tailed significance levels of 1 and 5%.

Numerical data included: one clinical observation (rearing), certain function tests (grip strength of forelimb and hindlimb, motor activity), body weight, body weight gain, food consumption, hematology, blood chemistry, and absolute and relative organ weights

Categorical data included: urinalysis parameters of pH, protein, glucose, ketones, and occult blood.

The following parameters were not evaluated statistically: clinical observations other than rearing, certain function tests (approach, touch, auditory, and tail pinch responses, and aerial righting reaction), ophthalmology, necropsy, or histopathology examinations.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were inspected twice a day during dosing and once per day during other periods.

1b. Clinical examinations: Detailed clinical examinations were conducted once before the dosing period started and once per week during dosing.

1c. Neurological evaluations: The following evaluations were performed once during week 12: sensory reactivity to stimuli, measurement of grip strength (with digital force gage), and motor activity [for 1 hour (10 min intervals)].

2. Body weight: Animals were weighed each week during the study and recovery periods.

3. Food consumption: Mean daily diet consumption was calculated from weekly data gathered for each rat.

4. Ophthalmoscopic examination: All animals' eyes were examined 3 days prior to the start of the study. Control and high dose group eyes were examined the final week of dosing.

5. Hematology and clinical chemistry: Rats were fasted overnight and blood was collected the next day on the scheduled necropsy days (at the end of the study and recovery periods) for hematology and clinical chemistry from all surviving animals. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	(Activated partial thromboplastin time (APTT))
X	Hemoglobin concentration (HGB)*	X	Leukocyte differential ratio*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB (MCH)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Platelet count*	X	Mean corpusc. volume (MCV)*
	Blood clotting measurements*	X	Reticulocyte ratio
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin*
X	Chlorine	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase (ALP)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. **Urinalysis***: Urine was collected from 5 males and 5 females from each group during the final week (day 89). The CHECKED (X) parameters were examined.

	Appearance*	X	Glucose
	Volume*	X	Ketones
	Specific gravity/osmolality*		Bilirubin
X	pH*	X	Blood/blood cells*
	Sediment (microscopic)		Nitrate
X	Protein*		Urobilinogen

* Optional for 90-day oral rodent studies

7. **Sacrifice and pathology**: All animals sacrificed on schedule (days 92 and 183) were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. Some organs were weighed in addition to being collected for histology (see XX in the table below). The tissues from all rats in the

control and high dose groups were examined microscopically from the sacrifices at day 92; livers, kidneys, and adrenals from the low and middle doses (day 92 sacrifice) as well as control and high dose rats (day 183 sacrifice) were also examined because lesions were seen in these organs at the highest dose during the dosing period.

The organs examined in this study are recommended by OECD TG 408; the nose, pharynx, and larynx were not collected for histology, probably because they are not recommended by OECD TG 408.

X	DIGESTIVE SYSTEM
	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
	Gall bladder (not rat)*
	Bile duct (rat)
X	Pancreas*
X	**RESPIRATORY**
X	Trachea*
X	Lung*
	Nose*
	Pharynx*
	Larynx*

X	CARDIOVASC./HEMAT.
X	Aorta*
XX	Heart*+
X	Bone marrow*
X	Lymph nodes*
XX	Spleen*+
XX	Thymus*+
X	**UROGENITAL**
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides*+
X	Prostate*
X	Seminal vesicles*
XX	Ovaries*+
XX	Uterus*+
X	Mammary gland*
X	Vagina

X	NEUROLOGIC
XX	Brain*+
X	Peripheral nerve (sciatic)*
X	Spinal cord (3 levels)*
X	Pituitary*
X	Eyes (optic nerve)*
X	**GLANDULAR**
XX	Adrenal gland*+
	Lacrimal gland
X	Parathyroid*
XX	Thyroid*
X	Harderian glands
X	**OTHER**
X	Bone (sternum and femur)
X	Skeletal muscle
X	Skin*
X	All gross lesions and masses*
X	Eyeballs

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
+ Organ weights required for rodent studies.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No treatment-related signs were seen.

2. **Mortality:** None

3. **Neurological evaluations:** Statistically increased rearing (0.02 mg/kg bw only at week 13 in males; 5 mg/kg bw only at week 8 in females) was not considered treatment related due to lack of a dose response.

B. BODY WEIGHT AND WEIGHT GAIN: In males, body weight gains at 0.5 mg/kg bw/day were decreased near the end of the study; differences were statistically significant ($p < 0.05$ on days 57 (19.7 vs. 25.3 in controls) and 64 (15.4 vs. 19.5)). However, there were no

statistically significant decreases in body weights. In addition, male body weight gains during recovery were higher than controls on several days and statistically significant only at day 106 (21.0 vs. 13.2 g; $p < 0.05$). Females did not show any statistically significant differences in body weights or body weight gains.

The minimal decreases in body weight gain did not lead to statistically significant decreases in body weight and are not more than 10% lower than controls. Thus, the body weight changes should not be considered biologically significant, which matches the authors' conclusions that there were no changes in body weights.

TABLE 2. Average body weights and body weight gains during 90 days of treatment [a]

Sex	Dose (mg/kg bw/day)	Body Weights		Body Weight Gains [Day 1 to 91]	
		Week 0 [Day 1]	Week 13 [Day 91]	g	% of control
Males	0	155.1 (6.1)	559.1 (31.9)	404	-
	0.02	155.1 (8.6)	561.3 (33.3)	406	101
	0.1	154.1 (7.7)	564.5 (28 3)	410	102
	0.5	153.9 (5.4)	537.5 (38.8)	384	95.0
Females	0	137.5 (6 2)	293.1 (17.2)	156	-
	0.5	137.9 (6.6)	315.5 (31.0)	178	114
	5	138.2 (5.7)	306.8 (25.8)	169	108
	50	137.7 (7.1)	297.0 (28.7)	159	102

[a] Data obtained from pages 204-215 in the study report.

C. FOOD CONSUMPTION:

In males, increased food consumption was seen on days 106, 113, and 120 of the recovery period at the highest dose ($p < 0.05$), which paralleled the increased body weight gains (day 106) during this time.

D. OPHTHALMOSCOPIC EXAMINATION:

There were no test-related changes observed in light reflex, anterior portion of the eye, optic media, or ocular fundus.

E. BLOOD ANALYSES:

1. **Hematology**: Statistically significant low hemoglobin concentration and hematocrit values were seen in males on day 92 at 0.1 and 0.5 mg/kg bw/day ($p < 0.01$), but not during recovery at the highest dose of 0.5 mg/kg bw/day [only the highest dose was measured during recovery]. During recovery (day 183), MCHC was lower in males at 0.5 mg/kg bw/day compared with controls ($p < 0.05$).

   In females, the only statistically significant effect was a decreased prothrombin time at the end of the study at 50 mg/kg bw/day ($p < 0.01$). However, at 50 mg/kg bw/day, two females had multiple changes in hematology (low RBC counts, hemoglobin concentration,

hematocrit); one of these females also had high MCH and MCV and markedly low platelet counts.

2. **Clinical chemistry:** *Males.* At the end of the study, males had increased ALT and ALP (both more than 2 times the control values) ($p < 0.01$) at 0.5 mg/kg bw/day. Total protein was higher at the highest dose, whereas albumin and the A/G ratio were higher at both the middle and high doses in males ($p < 0.01$).

In males, AST was also slightly increased at the end of the study (with unclear dose-response) but especially higher at 0.5 mg/kg bw/day vs. controls (205 vs. 66 U/L) at the end of recovery; none of these changes were statistically significant. Males also exhibited higher potassium at the end of the study at 0.5 mg/kg bw/day ($p < 0.01$). Inorganic phosphorus was lower only at the lowest dose (0.02 mg/kg bw/day) in males without a dose response.

Certain individual rats had very high values; two males had high AST and ALT values during dosing, and a single male had markedly high AST, ALT, potassium and GGT at the end of recovery.

Females. In females, statistically significant effects were seen only at 50 mg/kg bw/day. At the end of the study, total bilirubin was decreased ($p < 0.01$), albumin was increased ($p < 0.01$), and total protein and the A/G ratios were also increased ($p < 0.05$). However, at the end of recovery, total protein was *lower* than controls ($p < 0.05$).

Females also exhibited a trend towards dose-related increases in AST at the end of the study but without statistical significance; this effect was reversed during recovery (i.e., the high-dose AST value was lower than control values). Total cholesterol showed a decreasing trend with increasing dose (also without statistical significance).

A single female had markedly high AST and ALT values during dosing.

F. URINALYSIS: The authors report no abnormalities in any treatment group and the data also do not indicate any trends.

G. SACRIFICE AND PATHOLOGY:

1. Organ weight:

The liver and kidney weights (absolute and/or relative) were increased at the highest two doses (males) or highest dose (females) during the dosing period but not during recovery. Details are outlined in the table below:

Sex	Liver					Kidney				
	Dose (mg/kg bw/day)	*Dosing Period*		*Recovery*		Dose mg/kg	*Dosing Period*		*Recovery*	
		Absolute (g)	Relative (%)	Absolute (g)	Relative (%)		Absolute (g)	Relative (%)	Absolute (g)	Relative (%)
Male	0	13.7	2.60	16.2	2.53	0	3.28	0.626	3.55	0 556
	0.02	14.4	2.70	NE	NE	0.02	3.41	0.642	NE	NE
	0.1	17.5**	3.28**	NE	NE	0.1	3.59	0.675*	NE	NE
	0.5	22.8**	4.54**	15.6	2.48	0.5	3.49	0.694**	3.61	0 578
Female	0	7.11	2.58	7.98	2.50	0	1.75	0.634	1.97	0.618

	0 5	7.33	2.49	NE	NE	0.5	1 81	0.616	NE	NE
	5	7.56	2.63	NE	NE	5	1 77	0.617	NE	NE
	50	9.66**	3.43**	7.67	2.44	50	1.94*	0.691*	1 93	0.616

* P<0.05
** P < 0.01
NE = Not examined

In addition, the absolute epididymis weight was statistically significantly increased (1.68 vs. 1.52 g; $p < 0.05$) in males at the end of recovery. Also, uterus weight was lower at the end of recovery in the 50 mg/kg bw/day females (0.622 vs. 0.744 g; $p < 0.05$).

Absolute and relative thymus weights of males were statistically significantly increased only at the lowest dose (0.02 mg/kg bw/day).

2. **Gross pathology:** Generally, gross lesions were seen in the same organs that exhibited increased weights (primarily the liver and kidney).

In males, all 10 rats at 0.5 mg/kg bw/day and 9 at 0.1 mg/kg bw/day had enlarged livers, with one rat showing a yellowish patch on the liver at 0.5 mg/kg bw/day; this was seen in the rat that exhibited fibrosis - see next section. All 10 females at 50 mg/kg bw/day had enlarged livers. One mid-dose male rat (0.1 mg/kg bw/day) had a hepatodiaphragmatic nodule. At 0.5 mg/kg bw/day, one male had a small testis and one male had an enlarged epididymis.

No treatment-related changes were seen in rats at the end of the recovery period.

3. **Microscopic pathology:**

Numbers of rats with various liver lesions are summarized in the table below. The effects are not mutually exclusive (e.g., a single rat may exhibit hepatocyte hypertrophy as well as necrosis). Eosinophilic granular changes were observed in the cytoplasms of hepatocytes in all rats (except one male at 0.1 mg/kg bw/day) with hypertrophic hepatocytes.

Liver

Sex	Dose (mg/kg bw/day)	Dosing period [10 rats/sex]			Recovery [5 rats/sex]	
		Fibrosis (focal, minimal)	Hepatocyte hypertrophy (centrilobular, minimal)	Hepatocyte necrosis (focal, minimal)	Hepatocyte necrosis (focal, mild)	Hepatocyte vacuolation (perilobular, minimal)
Male	0	0	0	0	0	0
	0.02	0	0	1	NE	NE
	0 1	0	8	2	NE	NE
	0.5	1	10	4	2	0
Female	0	0	0	1	0	0
	0.5	0	0	0	NE	NE
	5	0	3	0	NE	NE
	50	0	10	0	0	1

NE = Not examined

Findings in kidneys (numbers of rats with effects) are noted in the table below and again, effects are not mutually exclusive.

Kidney

Sex	Dose (mg/kg bw/day)	Dosing period [10 rats/sex]			Recovery [5 rats/sex]		
		Basophilic tubule (focal; minimal)	Hyaline cast (focal, minimal)	Hypertrophy, proximal tubular epithelium (minimal)	Basophilic tubule		Hyaline cast
					Diffuse (mild)	Focal (minimal)	
Male	0	7	1	0	0	2	1
	0.02	4	0	0	NE	NE	NE
	0 1	4	0	7	NE	NE	NE
	0.5	8	1	10	1	1	1
Female	0	1	1	0	0	1	1
	0.5	0	1	0	NE	NE	NE
	5	2	2	4	NE	NE	NE
	50	3	2	8	0	2	2

NE = Not examined

Other findings: At the end of the study, other organs were examined only from high-dose rats and controls. A few histopathological findings were observed, primarily infiltration of inflammatory cells. In addition, retinal dysplasia was observed in one male and epididymal/testis changes seen in one rat. All incidences of inflammatory cell infiltration were of minimal severity (grade 1 on a scale of 1-4).

Gonads and Accessory Sex Organs

Sex	Dose (mg/kg bw/day)	Testis - atrophy	Epididymis [a]		Mammary gland – inflammatory cells in interstitium	Vagina – inflammatory cells in mucosa
			inflammatory cells in interstitium	decreased sperm, spermatic granuloma		
Male	0	0	0	0		
	0 5	1	1	1		
Female	0				0	0
	50				1	1

[a] Effects seen in two different rats, but the rat with decreased sperm/granuloma also had the atrophied testis

Other Tissues

Sex	Dose (mg/kg bw/day)	Adrenals	Lungs – foam cell accum	Salivary gland – inflamm cells	Lymph nodes (mandibular) – increased pigmentophages	Glandular stomach (increased leukocytes)	Eyes – retinal dysplasia
Male	0	0	2	0	0	0	0
	0 5	3 [a]	4	1	0	0	1
Female	0	0	0	0	0	0	0
	50	1 [b]	1	0	1	1	0

[a] Minimal diffuse hypertrophy of cortical cells in the glomerular zone

[b] Minimal accessory adrenocortical tissue

Although 2 males had ectopic thymic tissue in the thyroid at 0.5 mg/kg bw/day versus 1 in the control group, this effect was seen in 1 female control and no females at the highest dose, so there does not appear to be a dose response when data on sexes are combined [not shown in table]. No follicular cell hypetrophy was observed (in contrast to the 28-day study).

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: Several changes were considered by the authors to be unrelated to treatment because the individual values were within or almost within the range of historical controls. These effects included changes in hemoglobin, hematocrit and potassium (males), decreased MCHC (at 0.5 mg/kg bw/day in males at the end of recovery), or decreased prothrombin time and low bilirubin (females). The significant low total protein value in females at 50 mg/kg bw/day at the end of recovery was also not determined to be treatment-related because it was not seen after the end of the dosing period.

Because the low uterus and high epididymis weights were seen only at the end of the recovery period, the authors did not consider these to be treatment related. Also, due to the lack of dose response in increased thymus weights (effects seen at lowest dose only), differences in thymus weights were not considered to be treatment related by the study authors.

Other hematological and biochemical changes were considered to be treatment-related. These included decreases in RBC count, hemoglobin, hematocrit and platelet count and increases in MCV and MCH in females at 50 mg/kg bw/day, although no corresponding changes in bone marrow or spleen were seen. Increases in albumin, total protein and A/G ratio were considered to be related to other liver effects (e.g., hepatocyte hypertrophy).

Histopathological effects in the liver, kidneys, and adrenals were considered treatment-related. Based on eosinophilic granular changes in the cytoplasm of hepatocytes combined with the finding from the 14-day repeated-dose toxicity study[1] of enhanced cyanide-insensitive palmitoyl CoA β-oxidation induction, the authors considered the hepatocyte hypertrophy to be related to peroxisome proliferation. Liver necrosis was considered to be treatment-related except for 1 male at 0.02 mg/kg bw/day, because the incidence and severity were similar to controls (1 female control also exhibited hepatocyte necrosis). The increases in AST and ALT were considered to be related to hepatocellular necrosis. The diffuse hypertrophy in the adrenal glomerulosa cells in males at 0.5 mg/kg bw/day was also considered treatment-related.

Microscopic changes in other organs were not considered to be related to treatment because they were sporadically seen and did not show dose dependency.

The authors determined NOELs of 0.02 and 0.5 mg/kg bw/day for males and females, respectively. Although the authors didn't fully state the basis for these specific dose levels, they are assumed to be due to the combination of liver and kidney effects at 0.1 and 5 mg/kg bw/day in males and females, respectively.

B. REVIEWER COMMENTS: The authors' conclusions that liver, kidney and adrenals are primary target organs are reasonable. This information is consistent with the 28-day and 14-day studies and is indicated by the data from this study. However, the 'eosinophilic granular change in the cytoplasm of the hypertrophied hepatocytes' is not necessarily specific to peroxisome proliferation; it could signal other increased activities/granular bodies in the cytoplasm. It would be helpful to have more specific information from the current 90-day study on measures such as peroxisomal enzyme activity or peroxisome proliferation for comparison with the 14-day data,

[1] Study was submitted with the PMN, but was not found on documentum.

especially because the 14-day study was not located and details of the study could not be reviewed. However, the hepatocyte hypertrophy and the measurements in the 14-day study indicate that the PMN may cause peroxisome proliferation via PPARα.

Although some effects were discounted by the study authors because they were within or nearly within historical control ranges or because the effect was seen only at the end of recovery, certain of these effects in particular are likely to be treatment related (e.g., low hemoglobin, hematocrit, and RBC counts) based on their consistency with the 28-day study results. The significance of *decreased* prothrombin time is toxicologically unclear. However, it is possible that there is some relationship with treatment because changes in prothrombin time and APTT were observed in the 28-day study, although the effects were *prolonged* times vs. controls. Also, although the higher potassium values seen in males at 0.5 mg/kg-bw/day were within 1 standard deviation of the mean of the historical control data presented in Annex 6, potassium generally has a narrow clinical range (OECD, 2012), so the observed statistically significant difference may be important.

It is unclear whether effects on testis and epididymis at 0.5 mg/kg bw/day are treatment related. A publication available from [redacted] lists historical control data for Crl:CD(SD) rats ages 4-26 weeks old from short-term and juvenile studies ([redacted]. Data were reported for 34 studies conducted at three separate testing facilities and initiated between 2002 and 2009. The studies included gavage, i.v., intramuscular, percutaneous, and subcutaneous studies. In this publication, three incidences of testicular effects (tubular dilation, inflamed arterioles, necrosis) were seen in three separate gavage studies. Epididymal effects included several cases of inflammatory cell infiltration, three cases of exfoliated spermatogenic cells in two studies (gavage, i.v.) and two cases of hypospermia in two additional studies (both gavage).

Although the presence of retinal dysplasia is seen in one male at 0.5 mg/kg bw/day, the 28-day study showed this effect in one female at both 0 and 50 mg/kg bw/day. The [redacted] control data indicate retina dysplasia only in one female in an intravenous (i.v.) study (out of the 34 studies summarized). Three cases of retinopathy were also seen in males in these studies.

There may be some pattern of general inflammation related to the compound given the effects generally seen in single animals at the highest dose in other organs, albeit of minimal severity. It should be noted, however, that the pancreas and prostate showed similar (or greater) inflammatory infiltration in controls compared with dosed animals.

Overall, based on liver cell hypertrophy/necrosis, kidney cell hypertrophy and hematology and clinical chemistry changes, the LOAEL and NOAEL can be set at 0.1 and 0.02 mg/kg bw/day for males, respectively. In females, liver and kidney effects and non-statistical trends in enzyme levels suggest a LOAEL and NOAEL of 5 and 0.5 mg/kg bw/day, respectively. Although several effects observed are of minimal severity, given possible increasingly severe necrosis after removal of the test substance along with accompanying hematology and clinical chemistry changes, the chosen LOAELs and NOAELs are appropriate.

C. STUDY DEFICIENCIES:

One deviation from OECD TG 408 is the number of rats used per dose. Because different dose

levels were used for males and females (except for one dose), some doses only had 10 total rats, whereas OECD TG 408 specifies 20 rats/dose. For any effects that might show deviations from controls, differences in numbers of rats/dose will affect the statistical analysis of the results. However, based on consistency of effects compared with the 28- and 14-day studies, this deficiency is likely to be minor and the importance of evaluating the correct dosing for each sex should outweigh this deficiency.

SOURCES

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[redacted]